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IMMUNOHISTOCHEMICAL EVALUATION OF THE EXPRESSION OF PROSTATE TUMOR-ASSOCIATION MARKERS IN THE NUDE MOUSE HUMAN PROSTATE CARCINOMA HETEROTRANSPLANT LINES PC-82 PC-EW AND PC-EG
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ABSTRACT: The biotin-avidin immunoperoxidase assay was used to evaluate the expression of several prostate carcinoma-associated markers in formalin-fixed paraffin-embedded tissue sections of three human prostate nude mouse heterotransplant lines PC-82, PC-EW, and PC-EG. In addition to monoclonal antibodies to PSA and PAP, monoclonal antibodies to five other potentially useful markers for prostate carcinomas (TURP-27, Leu-7, 7E11-C5, PSP-19 and PD41) were tested. Tissues from two or more transplant passages were evaluated. The human prostate target antigens were found to be expressed by one or more of the three heterotransplant lines. The PC-82 and PC-EW lines were the most efficient in terms of expression of multiple prostate carcinoma-associated markers and percentage of tumor cells positive for a given prostate antigen. The staining pattern of each marker, in terms of staining intensity, number of tumor cells stained, and staining location, i.e., membrane, cytoplasmic, or ductal secretions, was similar to what has been observed in tissue sections from human prostate carcinomas. The lack of an appropriate model for evaluating the preclinical potential of these Mabs (especially TURP-27, PSP-19, and PD41) makes the findings of this study of considerable importance, and suggests that these human prostate xenografts may be useful models for exploring the diagnostic and therapeutic potential of these anti-prostate carcinoma monoclonal antibodies.

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Immunohistochemical Evaluation of the Expression of Prostate Tumor-Association Markers in the Nude Mouse Human Prostate Carcinoma Heterotransplant Lines PC-82, PC-EW, and PC-EG

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The biotin-avidin immunoperoxidase assay was used to evaluate the expression of several prostate carcinoma-associated markers in formalin-fixed paraffin-embedded tissue sections of three human prostate nude mouse heterotransplant lines PC-82, PC-EW, and PC-EG. In addition to monoclonal antibodies to PSA and PAP, monoclonal antibodies to five other potentially useful markers for prostate carcinomas (TURP-27, Leu-7, 7E11-C5, PSP-19, and PD41) were tested. Tissues from two or more transplant passages were evaluated. The human prostate target antigens were found to be expressed by one or more of the three heterotransplant lines. The PC-82 and PC-EW lines were the most efficient in terms of expression of multiple prostate carcinoma-associated markers and percentage of tumor cells positive for a given prostate antigen. The staining pattern of each marker, in terms of staining intensity, number of tumor cells stained, and staining location, i.e., membrane, cytoplasmic, or ductal secretions, was similar to what has been observed in tissue sections from human prostate carcinomas. The lack of an appropriate model for evaluating the preclinical potential of these Mabs (especially TURP-27, PSP-19, and PD41) makes the findings of this study of considerable importance, and suggests that these human prostate xenografts may be useful models for exploring the diagnostic and therapeutic potential of these anti-prostate carcinoma monoclonal antibodies.

Key words: prostate cancer, nude mice, monoclonal antibodies, prostate carcinoma-associated markers

INTRODUCTION

The availability of human prostate carcinoma models that express tumor-associated markers has been disappointing. So far only three cultured prostate cell lines,

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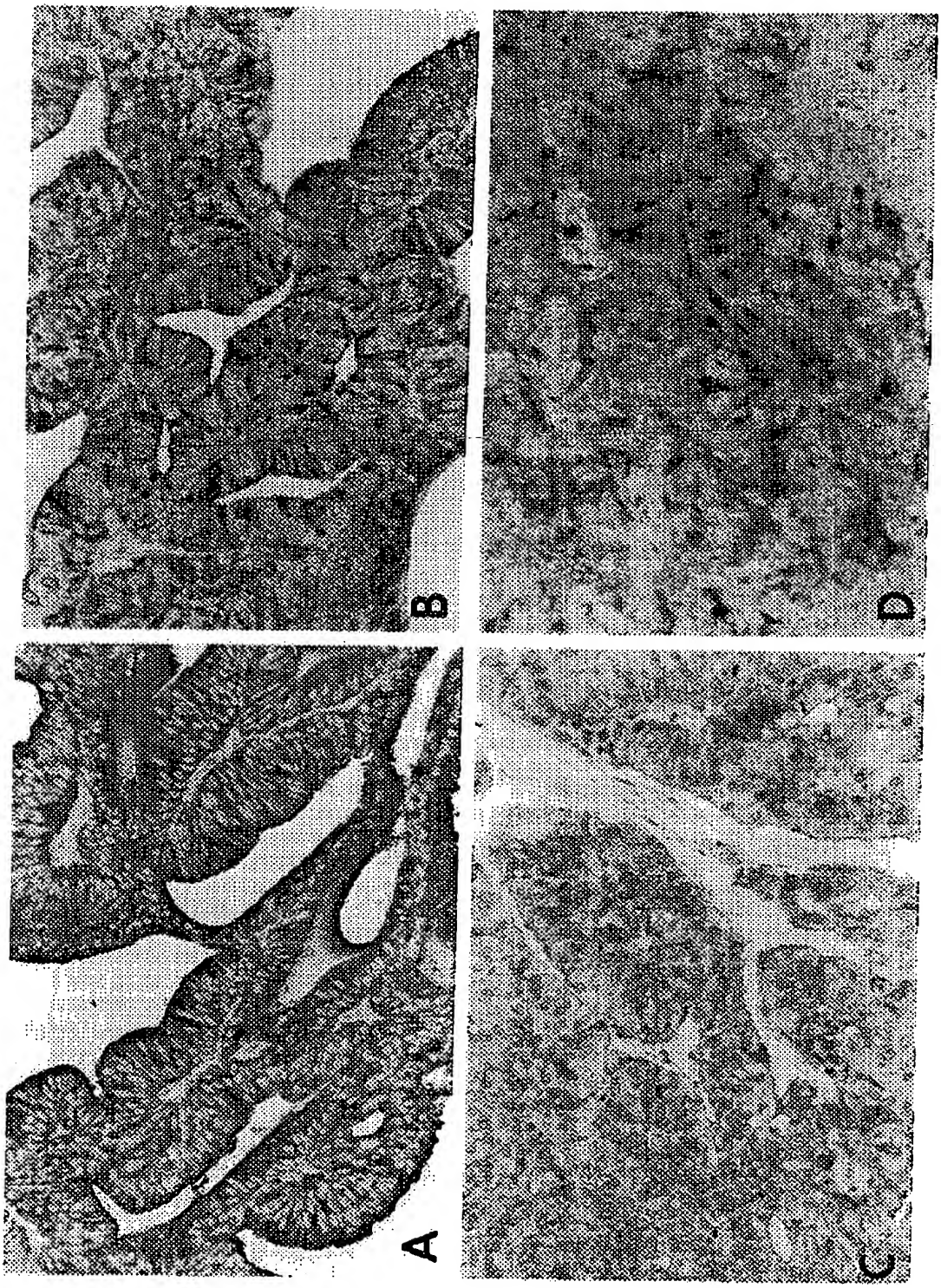

Fig. 1.

DU145, PC3, and LNCaP, have been established and extensively characterized as to their origin, hormone dependence, malignancy, genetic constitution and expression of tumor markers [1–4]. All three cultured prostate carcinoma lines were derived from metastatic tissues and only the LNCaP line reproducibly secretes a prostate carcinoma-associated marker [4]. The establishment of cultured prostate carcinoma cell lines has been extremely difficult, most likely due to their fastidious growth requirements and yet-to-be identified growth factors required to establish and maintain growth in artificial medium. Recently we described the establishment and preliminary characterization of a new prostate cell line, PPC-1, derived from a primary carcinoma [5]. To the best of our knowledge this is the first prostate tumor cell line to be established in culture from a primary prostate adenocarcinoma. Whether the PPC-1 line expresses prostate tumor markers and can be used as a model for evaluating the potential clinical utility of anti-prostate carcinoma monoclonal antibodies remains to be determined.

Similarly, attempts to initiate human prostate tumor heterotransplants in immunosuppressed animals have been met with the same degree of failure. One group of investigators has, however, been successful in establishing three transplantable human prostate carcinoma lines in nude mice [6–11]. These lines are designated PC-82, PC-EW, and PC-EG. PC-82 and PC-EG were derived from primary prostate carcinoma tissue removed at surgery and PC-EW was derived from a lymph node metastasis. All three xenograft lines retain the histopathologic characteristics of the patient's original tumor, are androgen dependent, and secrete the prostate tumor markers prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA) [12,13]. These prostate carcinoma heterotransplant lines represent the most frequently occurring types of human prostate cancer and have proven to be very useful in studying the in vivo effect of endocrine manipulation on tumor growth and on the expression and secretion of PAP and PSA [6,8,10,11].

Our laboratory has identified monoclonal antibodies (Mabs) recognizing antigenic markers on prostate carcinoma cells that could have potential clinical application [14,15,17]. However, several of the target antigens recognized by these Mabs do not appear to be expressed on cultured prostate or non-prostate tumor cell lines. Therefore, identifying a model system or systems for assessing the pre-clinical utility of these monoclonal antibodies and tumor markers is of extreme importance. The objective of the present study was to determine if the transplantable prostate tumor lines PC-82, PC-EW, or PC-EG, expressed prostate carcinoma-associated markers in addition to PAP and PSA.

Fig. 1. Immunoperoxidase staining of PC-82 and PC-EW tissue sections with Mabs PAP and PSA. **A:** PC-82 stained with Mab PAP. Note intense cytoplasmic staining of most of the tumor cells. **B:** PC-82 stained with Mab PSA showing intense cytoplasmic staining with some focal staining of the tumor cells. **C:** PC-EW stained with Mab PAP shows moderately diffuse cytoplasmic staining of the majority of the tumor cells and no staining of mouse connective tissue. **D:** PC-EW stained with Mab PSA showing a weak to moderately diffuse cytoplasmic staining in most of the tumor cells. A few of the tumor cells also show intense focal staining. Original magnification: ×100 **(A, B)**; ×200 **(C,D)**.

TABLE I. Characteristics of the Nude Mouse Human Prostat Carcinoma Heterotransplant Tum r Lines

	PC-82	PC-EW	PC-EG
Origin	Prostatectomy	Lymphadenectomy	TURP[a]
Histopathology of patients' tumor	Moderate to poorly differentiated	Poorly differentiated	Pooly differentiated
Transplant passage tested	22, 28, 32, 33, 34	17, 18	4, 5
Histopathology of tumor xenograft	Moderately differentiated with cribriform pattern	Moderately (cribriform pattern) to poorly differentiated	Poorly differentiated to undifferentiated
Doubling time	18 days	10 days	15 days
Androgen dependence	Yes	Yes	Yes
Take rate of tumor grafts	90%	90%	70%
Serum PAP	Yes	Yes	Yes
Serum PSA	Yes	Yes	Yes

[a]Transurethral resected prostate.

MATERIALS AND METHODS

Tissue Specimens

The origin of the human prostate tumor tissue and the establishment and maintenance of the PC-82, PC-EW, and PC-EG lines by serial transplantation in nude mice have been previously described [6,9,10]. Formalin-fixed paraffin-embedded blocks of the PC-82, PC-EW, and PC-EG tumors from different transplantation passages were examined in this study. The major characteristics of each of the heterotransplant lines are presented in Table I.

Monoclonal Antibodies

The Mabs used in this study are listed in Table II. All Mabs selected for this study were previously shown to bind their respective target antigens in formalin-fixed paraffin-embedded human prostate carcinomas.

Mabs to prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) used in this study were produced in our laboratory in Balb/c mice following the hybridoma protocol previously described [14,15]. These Mabs were generated by using a purified preparation of the PSA and PAP antigens isolated from pooled normal seminal plasma [16]. The PSA Mab has the IgG1 (k) isotype, whereas the PAP Mab is of the IgG2a (k) isotype. The selected Mabs were determined to be prostate specific following extensive screening of normal and malignant human tissues by immunoperoxidase staining. Western blot analysis following SDS-PAGE of

Fig. 2. Prostate carcinoma heterotransplants stained with Mabs TURP-27 and Leu-7. **A:** PC-82 stained with Mab TURP-27 showing moderate to intense cytoplasmic and membrane staining of the majority of the tumor cells. **B:** PC-EW stained with Mab TURP-27 showing intense cytoplasmic, membrane, and focal staining of the tumor cells. **C:** PC-EW stained with Mab Leu-7. Intense cytoplasmic staining of the majority of the tumor cells with intense focal staining of some tumor cells can be seen. **D:** PC-EG stained with Mab TURP-27 showing dark membrane or focal staining in some of the tumor cells. Original magnification: ×100 **(A)**; ×200 **(B,C,D)**.

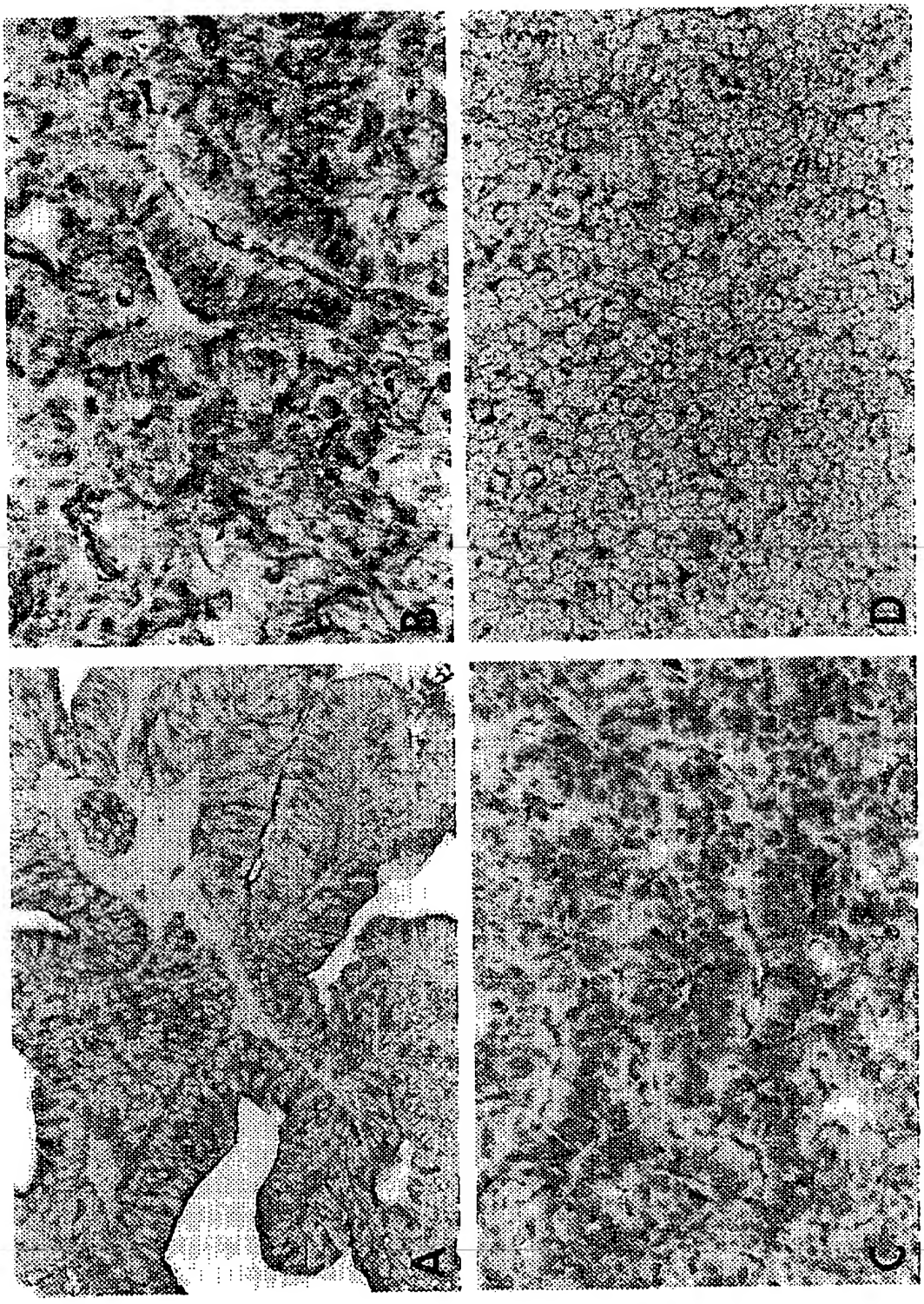

Fig. 2.

TABLE II. List f Anti-Prostate M noclonal Antibodies

Monoclonal antibody	Isotype	Specificity	Antigen	Reference
TURP-27	IgG3	Prostate (N, B, T)[a] myelinated nerves	Glycoprotein complex (310, 250, 180, 140, 120, 95, 69, 35 kD)	[14,27]
Leu-7	IgM	Prostate (N, B, T) CNS tissues, neural ectoderm tissues	Glycoprotein complex (205, 170, 135, 110, 85, 65, 55 kD)	[17,29]
PSA	IgG1	Prostate (N, B, T)	Protein (36 kD)	Wright (unpublished)
PAP	IgG2a	Prostate (N, B, T) adrenal gland	Protein (100 kD, 50 kD subunit)	Wright (unpublished)
PSP-19	IgG1	Prostate (N, B, T) respiratory tissues (?), Stomach (?)	Protein (16 kD)	[15]
PD41	IgG1	Prostate (T)	Mucin-like glycoprotein (> 400 kD)	Wright (unpublished)
7E11-C5	IgG1	Prostate (N, B, T) skeletal muscle?	Protein (?) (100 kD)	[20,26]

[a]N = normal, B = benign, T = tumor.

purified PSA and PAP, seminal plasma, and prostate tissue extracts showed that the PSA Mab recognized only a 36 kD protein while the PAP Mab bound only to the 50 kD subunit of PAP, thus confirming the specificity of these two Mabs.

The prostate secretory protein (PSP) Mab (PSP-19) used in this study was produced and used as described in a recent report [15]. Briefly, the 16 kD PSP molecule was purified from pooled seminal plasma and used to immunize Balb/c mice. The specificity of this IgG1 (k) Mab for prostate tissues was determined as described above for Mabs PSA and PAP.

The production and tissue specificity of the TURP-27 and Leu-7 Mabs have been previously described [14,17]. Mab TURP-27 is an IgG3 antibody which recognizes a complex of glycoproteins ranging from 310 kD to 35 kD. The TURP-27 antigen is maximally expressed on benign and malignant prostate carcinomas with some cross reactivity with myelinated nerve fibers. The Leu-7 Mab is an IgM antibody produced by the HNK-1 hybridoma (American Type Culture Collection) which also recognizes several glycoprotein species bearing the HNK-1 epitope (Table II) [27]. The Leu-7 Mab has a broader non-prostate tissue reactivity, binding to a variety of neuroectoderm-derived tissues including melanomas, neuroblastomas, and small-cell lung carcinomas [18,19].

PD41 is an IgG1 Mab that we have recently isolated in our laboratory following the immunization of Balb/c mice with a tissue extract prepared from a poorly differentiated prostate carcinoma. Following exhaustive specificity testing, this Mab was found to react to 65% of the prostate carcinomas tested. It does not bind to normal or benign prostate tissues or a large variety of normal and malignant non-prostate tissues. By Western blot analysis, Mab PD41 was shown to bind to a high molecular weight (>400 kD) mucin-like glycoprotein. (Details of the production and specificity of this novel Mab will be the subject of a separate publication.)

The production and specificity of the 7E11-C5 (IgG1) Mab were first described by Horoszewicz [20]. Briefly, the Mab was produced by immunizing mice with the established LNCaP prostate carcinoma cell line. Like PSA, this Mab appears to be

restricted to prostate epithelial cells, including normal and benign as well as malignant cells, although some cross reactivity to skeletal muscle has been noted [26] (Wright et al., unpublished observations). Preliminary studies in our laboratory have shown, by Western blot analysis, that the 7E11-C5 Mab recognizes a distinct 100 kD protein antigen [28]. Partially purified ascites fluid containing 5 mg/ml of the 7E11-C5 Mab was kindly provided as a gift from CYTOGEN Corporation, Princeton, New Jersey.

Immunoperoxidase Staining

Tissue reactivity of the Mabs was examined on 5 μm sections of formalin-fixed paraffin-embedded tissues by the avidin-biotin complex immunoperoxidase assay by using a Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA) as previously described [14,15,17,21]. Tissue reactivity was scored by calculating the percentage of cells positive in each of four major quadrants in each specimen as well as scoring the intensity of the staining reaction using a scale of 0 (absence of staining) to 4 (most intense staining). The staining reactivity was scored independently by two different investigators.

RESULTS

The immunoreactivity of the panel of anti-prostate carcinoma-associated Mabs with formalin-fixed tissue sections of the PC-82, PC-EW, and PC-EG heterotransplants is shown in Table III. None of the Mabs were found to react to any mouse tissue (data not shown), although the IgM control Mab and Leu-7, also an IgM Mab, showed pale background staining of the xenograft tumor cells and mouse connective tissues. As expected all three human prostate heterotransplant lines expressed PAP and PSA, although neither PAP nor PSA was detected in tissue sections from the fifth transplant passage of the PC-EG line. Furthermore, the highest staining intensity and greatest number of cells expressing these two prostate markers were found in the PC-82 and PC-EW lines, and this expression appeared to remain fairly consistent from passage to passage (Table III). The pattern of staining reactivity was predominantly cytoplasmic for both PAP and PSA. There was, however, some intense focal staining at the luminal edge or between epithelial cells with all passages of PC-82 and with passage 18 of the PC-EW line using the PSA antibody (Fig. 1A and B).

Mabs TURP-27 and Leu-7 were found to bind to a high percentage (90%) of tumor cells in the PC-82 and PC-EW lines and to a lower percentage of tumor cells in the PC-EG line (Table III). Both cytoplasmic staining and membrane staining were observed with both Mabs (Fig. 2A–D).

The 7E11-C5 Mab demonstrated moderate staining reactivity in approximately 50% of the tumor cells in sections of the PC-82 line and passage 17 of the PC-EW line, whereas only 15–20% of the tumor cells in sections of passage 18 of the PC-EW line and both passages of the PC-EG line were stained (Table III). The tumor cells in all lines exhibited either membrane or focal staining; however, considerable staining was also noted within the lumen of ducts and interductal spaces (Fig. 3A and B). The latter staining did not appear to be an artifact since the IgG1 isotype-matched control Mab did not result in staining of the cells or luminal contents (Fig. 3C).

Prostate secretory protein (PSP) was found to be expressed by a few scattered tumor cells in both passages of the PC-82 and PC-EW lines but staining was not

TABLE III. Immunoperoxidase Staining of F rmalin-Fixed Tissue Sections of The PC-82, PC-EW, and PC-EG Prostate Carcinoma Heterotransplant Lines With a Panel of Anti-Prostate Monoclonal Antibodies

Monoclonal antibody	Tumor line	Passage[a] number	Staining[b] intensity	% Positive cells
TURP-27	PC-82	22	3–4+	86
	PC-82	28	3–4+	83
	PC-82	32	3+	100
	PC-82	33	3–4+	95
	PC-82	34	3–4+	92
	PC-EW	17	2–3+	86
	PC-EW	18	2–3+	93
	PC-EG	4	3–4+	75
	PC-EG	5	1–2+	10
PSA	PC-82	22	3–4+	85
	PC-82	28	3–4+	81
	PC-82	32	3+	90
	PC-82	33	3–4+	95
	PC-82	34	3–4+	95
	PC-EW	17	3+	92
	PC-EW	18	3–4+	90
	PC-EG	4	1–3+	55
	PC-EG	5	0	0
PAP	PC-82	22	3–4+	87
	PC-82	28	3–4+	90
	PC-82	32	3–4+	100
	PC-82	33	3–4+	100
	PC-82	34	3–4+	98
	PC-EW	17	2–3+	95
	PC-EW	18	3–4+	100
	PC-EG	4	1–3+	40
	PC-EG	5	0	0
PSP-19	PC-82	32	2+	15
	PC-82	33	2+	10
	PC-EW	17	2+	25
	PC-EW	18	2+	20
	PC-EG	4	0	0
	PC-EG	5	0	0
Leu-7	PC-82	32	3–4+	90
	PC-82	33	3–4+	90
	PC-EW	17	2–3+	82
	PC-EW	18	3–4+	90
	PC-EG	4	2–4+	73
	PC-EG	5	2–3+	68
PD41	PC-82	32	2+	2
	PC-82	33	0+	0
	PC-EW	17	3–4+	23
	PC-EW	18	0	0
	PC-EG	4	0	0
	PC-EG	5	0	0
7E11-C5	PC-82	32	2–3+	56
	PC-82	33	1–2+	45
	PC-EW	17	2+	42
	PC-EW	18	1+	15
	PC-EG	4	2+	20
	PC-EG	5	2+	15

[a]Number refers to transplantation passage from donor animal to recipient animal.
[b]Staining: 0 = no staining, 1+ = weak staining, 2+ = moderate staining, 3+ = strong staining, 4+ = very strong staining.

observed in sections of the PC-EG line (Table III). Moderate to strong staining intensity of the tumor cells was confined to the cytoplasm (Fig. 4A and B).

Mab PD41 did not react with its target antigen in the PC-82 heterotransplant, passage 33, or with either passage of the PC-EG line, while only 2% of the cells in sections of PC-82, passage 32, were stained (Table III). A greater percentage (23%) of tumor cells in passage 17 of the PC-EW line was stained with this Mab. However, none of the tumor cells in passage 18 of the PC-EW line were found to be stained with PD41 (Table III). The staining of the tumor cells with PD41 was predominantly confined to the cytoplasm; however, very intense staining of the luminal contents of most ducts was also noted (Fig. 5A and B). This pattern of staining in the nude mouse heterotransplants was very similar to the staining pattern observed in human prostate carcinoma tissue sections (Fig. 5C and D). As was observed with the 7E11-C5 Mab, the secretions or concretions did not appear to be staining artifacts since the IgG1 isotype control Mab did not stain any of the tumor cells or luminal contents. In all cases where there was no staining of tumor tissues, the experiments were repeated several times and the same negative results were obtained.

A comparison of the percent of tumor cells stained in tissue sections of the human prostate nude mouse heterotransplant lines PC-82, PC-EW, and PC-EG by the seven Mabs tested in this study is presented in Figure 6. Although at least one prostate carcinoma heterotransplant line was found to express a particular prostate antigen, the PC-EW and PC-82 appeared to express all seven tumor markers. Though five of the seven prostate tumor antigens were detected in tissue sections of the PC-EG tumor, a smaller number of tumor cells expressing these markers were usually observed.

DISCUSSION

This study confirms the expression of human PAP and PSA by the prostate carcinoma heterotransplant lines PC-82, PC-EW, and PC-EG, as well as demonstrating that these lines also express other prostate tumor-associated antigens. Since most of these antigens are not expressed by cultured tumor cell lines, this is an important finding as these heterotransplant lines may be useful as models for determining the pre-clinical application of these prostate carcinoma markers.

Some of the problems we have encountered in attempting to determine the clinical usefulness of new prostate carcinoma-associated markers and monoclonal antibodies are outlined below. A discussion of these problems will illustrate the potential value that these heterotransplant lines, especially the PC-82 and PC-EW lines, may have in determining the diagnostic, prognostic, and therapeutic utility of prostate tumor antigen-Mab systems.

Mab TURP-27 recognizes a series of novel sialoglycoproteins present in more than 95% of all prostate carcinomas which are produced by prostate ductal epithelial cells [14] (Wright et al., unpublished). Unfortunately this tumor-associated antigen is not expressed by cultured prostate carcinoma cell lines PC3, DU145, and LNCaP or any of over 40 non-prostate malignant cell lines tested [14] (Wright et al., unpublished). The high expression (90% or greater) of this TAA by the PC-82 and PC-EW lines suggests that these transplant lines may be excellent models in which to develop radioimmunologic imaging strategies and for determining the potential effectiveness of TURP-27 antibody-conjugates for prostate carcinoma therapy. We recently reported that ^{125}I-labeled TURP-27 specifically targeted human prostate carcinoma

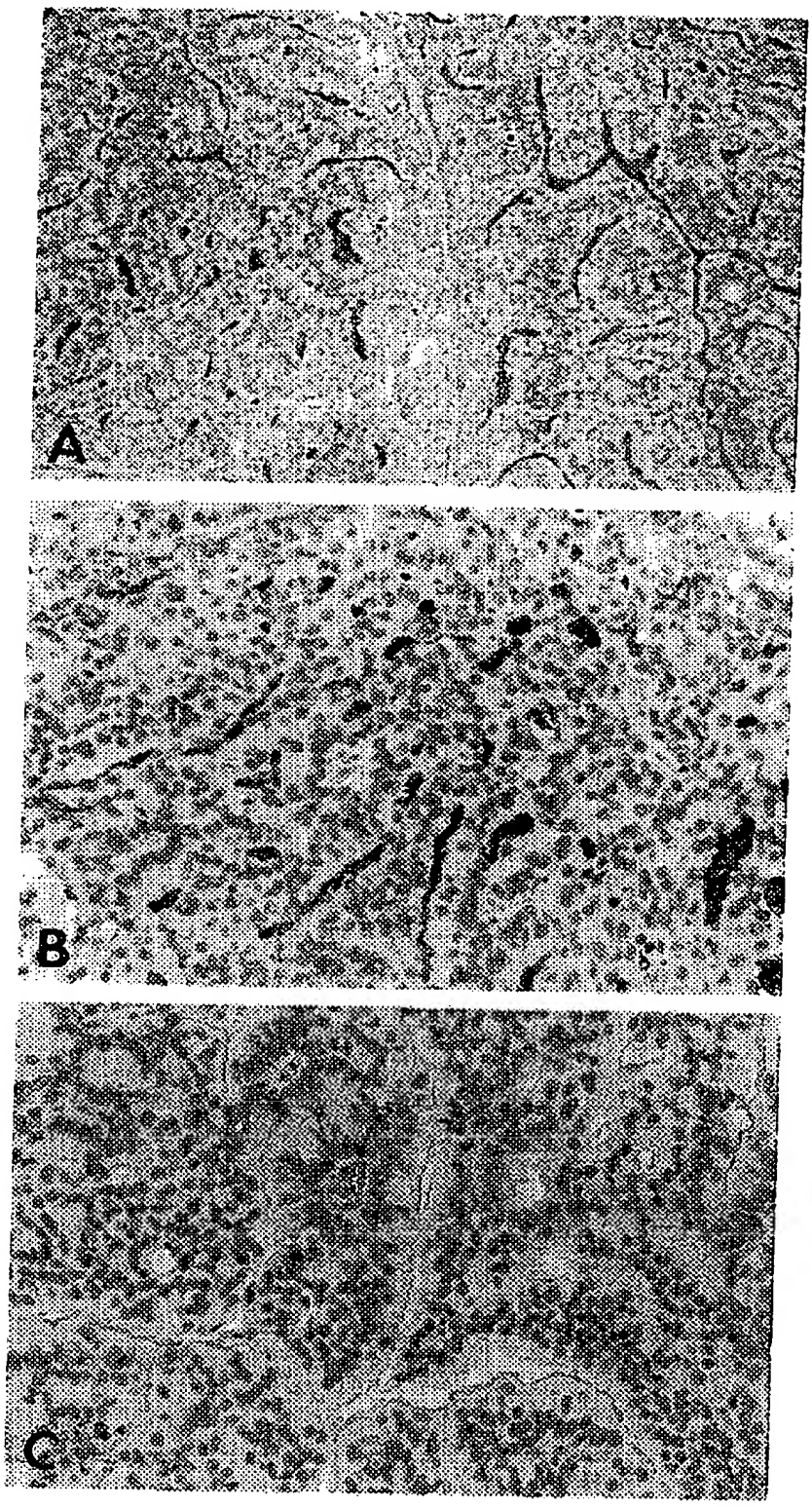

Fig. 3.

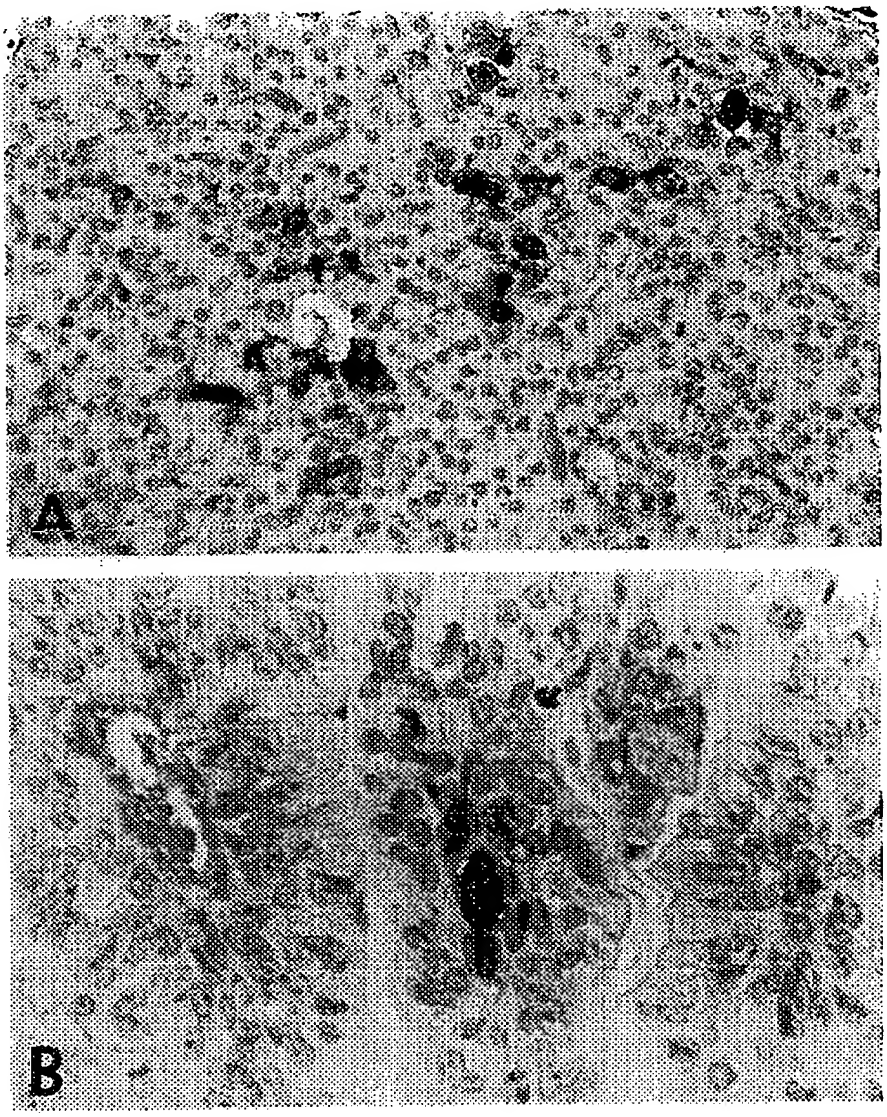

Fig. 4. PC-EW prostate carcinoma heterotransplant stained with Mab PSP. Both sections **A** and **B** show only a few individual tumor cells intensely stained while the majority of the neoplastic human tumor cells are unstained with Mab PSP. Original magnification: ×200 **(A)**; ×400 **(B)**.

Fig. 3. Immunoperoxidase staining of PC-EW with Mab 7E11-C5. **A:** This section shows intense staining of the luminal contents of ducts and in the interductal spaces. **B:** A higher magnification shows both intense staining of the luminal secretions and some of the isolated tumor cells. **C:** This section of PC-EW is stained with the IgG1-negative control Mab. No staining of the either the luminal contents or tumor cells can be seen. Only the nuclei are stained by the hematoxylin counterstain. Original magnification: ×100 **(A)**; ×200 **(B,C)**.

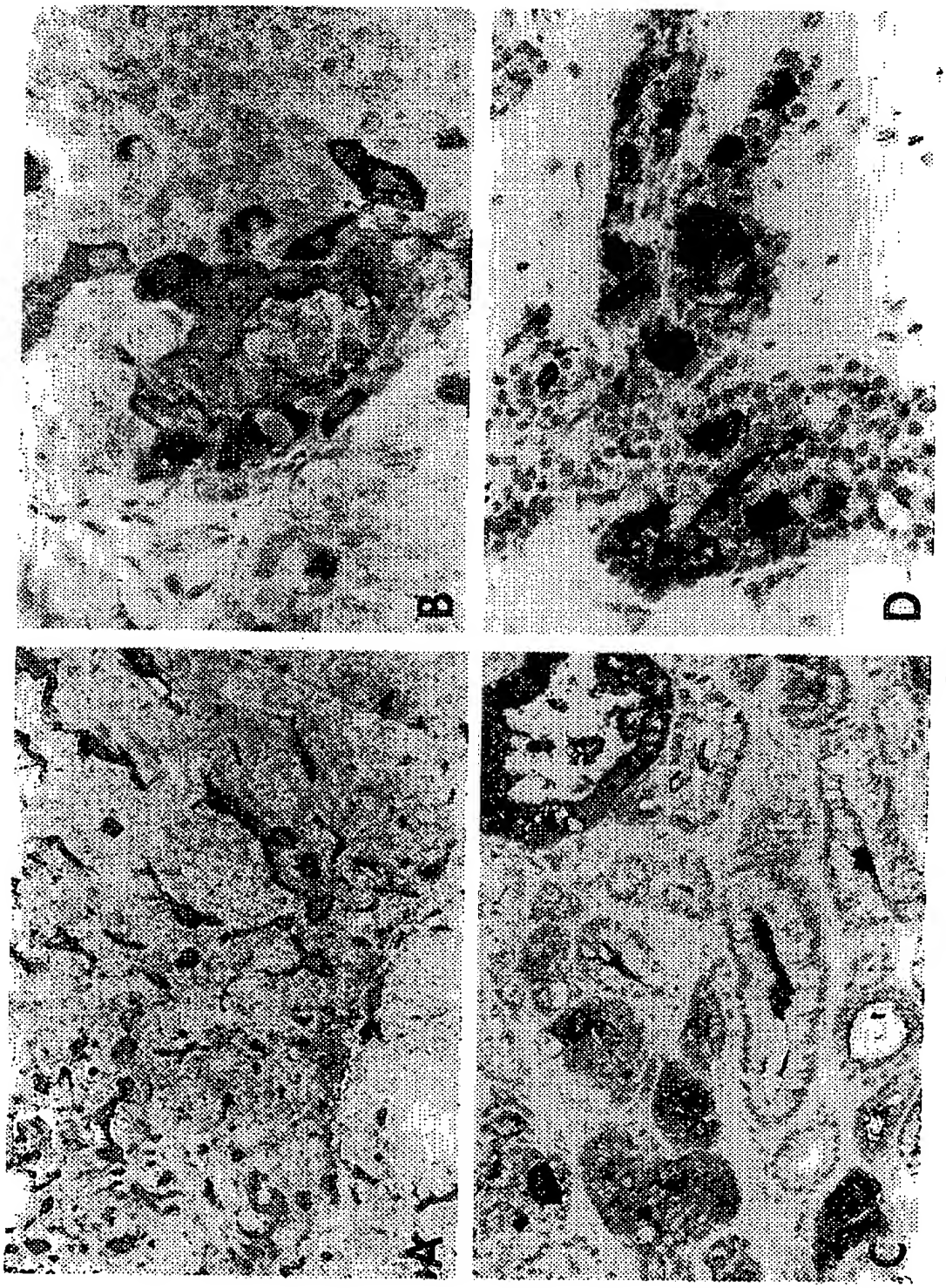

Fig. 5.

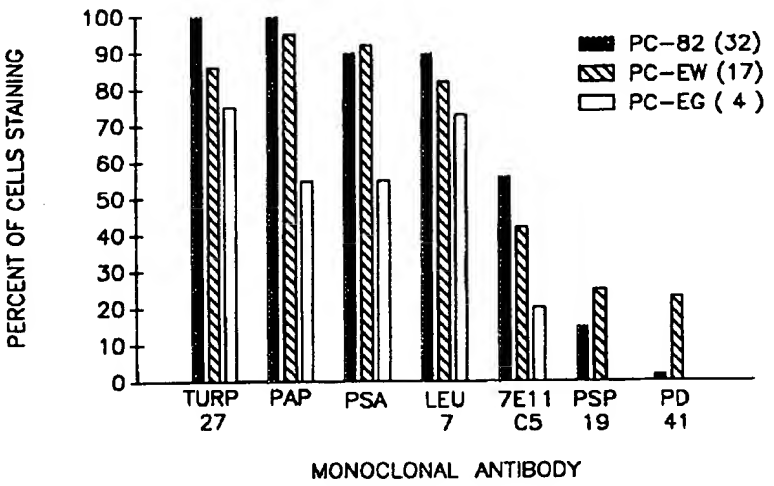

Fig. 6. Comparison of the percentage of tumor cells expressing the target antigens recognized in the three heterotransplant lines by the panel of anti-prostate tumor-associated monoclonal antibodies. The number in ( ) is the passage number tested.

implanted under the renal capsule of mice [22]. Although the mouse subrenal capsule model demonstrated the potential use of isotope-conjugated TURP-27 for targeting prostate carcinomas, the model is short term (3 days), such that pharmacokinetics and dosimetry determinations are almost impossible to perform. Furthermore, because of the short viability of the human prostate tumor explant, the subrenal capsule model is not useful for determining the therapeutic potential of conjugated antibodies.

A similar situation exists for the prostate tumor-associated marker recognized by Mab PSP-19. This Mab detects a 16 kD polypeptide antigen, designated prostate secretory protein or PSP. We and other investigators [15,23,24] have found that this prostate marker is maximally expressed on normal prostate ductal epithelial cells as well as benign and well-differentiated prostate carcinomas. The expression of PSP, however, decreases as the tumor becomes less differentiated. Therefore, the low expression of PSP in the heterotransplant lines was expected since these tumors are all moderately to poorly differentiated carcinomas. This marker, like TURP-27, is not expressed by any of the cultured prostate tumor lines tested [25] (Wright et al., unpublished), even though the PSP gene is present in the PC-3 cell line [25]. These data suggest that as the tumor becomes more undifferentiated the gene encoding PSP becomes suppressed. The low expression of PSP in the PC-EW line is similar to what

Fig. 5. Immunoperoxidase staining of the PC-EW prostate carcinoma heterotransplant line and two human prostate adenocarcinomas with Mab PD41. **A:** PC-EW section showing intense staining in the lumen of ducts and in the cytoplasm of some tumor cells. **B:** A higher magnification of a tissue section stained with PD41 showing the intense cytoplasmic staining of some of the neoplastic epithelial cells and luminal contents of a single duct. **C:** A section of a surgically removed well-differentiated human prostate adenocarcinoma stained with PD41. Both the intense staining of the tumor cells and ductal secretions are similar to that observed in the PC-EW heterotransplant line. **D:** A poorly differentiated human prostate adenocarcinoma stained with PD41. Again intense staining of some tumor cells and luminal contents can be seen. Original magnification: ×100 **(A,C):** ×400 **(B,D).**

has been observed in immunoperoxidase-stained tissues of surgically removed, poorly differentiated human prostate carcinomas [15]; therefore, PC-EW may be a useful model for the pre-clinical evaluation of PSP-19.

A Mab designated 7E11-C5 was recently described by Horoszewicz et al. [20] and appears to detect a new prostate-associated marker expressed on prostate epithelial cells. The antigen recognized by this Mab is expressed by the cultured LNCaP cells (the immunogen used to produce the 7E11-C5 Mab) and LNCaP nude mouse xenograft tumors. However, the fastidious in vitro growth of the LNCaP cultured cell line and the poor take rate and long doubling time of the LNCaP xenografts might hinder some pre-clinical studies of this antigen-Mab system. Our results would suggest that either PC-82 or PC-EW might be an acceptable model for further study of this interesting Mab and its target antigen.

Unlike PAP, PSA, PSP, TURP-27, Leu-7, and 7E11-C5, the PD41 Mab appears to recognize a mucin-like antigen restricted to prostate carcinomas. By immunoperoxidase analysis, PD41 binds the antigen in 65% of prostate carcinomas [Wright, et al., unpublished]. The antigen is expressed in the cytoplasm, usually in cells scattered throughout the tissue specimen, and is often observed in the luminal border of the ductal epithelial cells and as a secretion in the lumen of prostatic ducts. A similar heterogeneous staining pattern in the PC-EW line suggests that this line would be a useful model for pre-clinical studies and as a source of antigen for biochemical and immunochemical characterization. Both the PD41 and 7E11-C5 Mabs have shown a more intense staining reaction in unfixed frozen sections of human prostate carcinomas. Fresh or frozen heterotransplant tumors were not available for this study; therefore the potential enhanced staining in unfixed heterotransplant tumors remains to be determined.

There is increasing evidence that the marked heterogeneity of solid tumors, including prostate carcinomas, makes it necessary to use a panel of anti-prostate tumor Mabs, each recognizing a different subpopulation of tumor cell, in order to achieve successful antibody-directed therapy. The prostate carcinoma heterotransplant lines, especially PC-82 and PC-EW, were shown in this study to express several different prostate tumor markers. Therefore, it could be anticipated that these lines would be especially significant models for studying the biology, immunopharmacokinetics, interrelationship of marker expression, and therapy using multiple antibody-conjugates.

In summary, we have demonstrated by immunohistochemistry the expression of several different prostate TAA markers, in addition to PSA and PAP, in tissue sections of the prostate carcinoma nude mouse heterotransplant lines PC-82, PC-EW, and PC-EG. The fact that these heterotransplant lines, in general, retain the histological morphology of the patient's original tumor, have a high percent transplantable take rate, and express multiple prostate carcinoma-associated markers makes them potentially useful as models for determining the pre-clinical utility of anti-prostate TAA Mabs.

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